

## **PROTEIN AND LIPID SOURCES FOR USE IN AQUAFEEDS AND ANIMAL FEEDS AND A PROCESS FOR THEIR PREPARATION**

This application is a Continuation-In-Part application and claims priority on Canadian patent application serial number 2,351,903 filed on June 26, 2001 and International Patent Application Number PCT/CA01/00663 filed on May 8, 2001 which claims priority on Canadian patent application serial number 2,334,745 filed on February 13, 2001 and on United States serial number 09/566,728, which was allowed on November 2, 2001.

### **Field of the Invention**

The present invention relates to a novel process for the production of nutritionally upgraded protein and lipid sources for use in aquafeeds and other animal feeds. More specifically, the present invention relates to a process involving the co-processing of animal offal(s) with oilseed(s); the invention also relates to products produced thereby. Other embodiments of the present invention relate to novel oilseed protein concentrates and novel protein and lipid-rich oilseed meals, as well as novel oilseed oils produced using the process of the present invention.

In addition, the invention relates to cold pressed plant oils suitable for organic human foods, as well as products for use as components in organic fertilizers, both produced by the process of the invention.

### **Background of the Invention**

20 Feed accounts for on average 35-60% of the operating costs of salmon farms and it represents the largest cost in the culture of other carnivorous aquatic species. Moreover, the protein sources presently used account for the majority of the feed cost. Accordingly, salmon farming profitability is marginal in many regions. Hence, there is a need to reduce production costs and improve the market value of the farmed product.

Currently, aquatic feeds contain high levels of fish meal and oil, which are mostly imported, to produce a protein-rich and sometimes lipid-rich (e.g. salmon diets) aquatic feed. However, as noted hereinabove, such fish meal and oil can be very expensive and

this will be especially true in the future due to progressively increasing demands that are being placed on the finite global supplies of fish meal and oil. Hence, alternative economical sources of protein and lipid are required.

One known approach is to use less expensive plant protein sources in aquafeed that have been specially processed so that they are in the form of nutritionally upgraded protein meals, concentrates, and isolates. These may be used either singly or in combination with rendered animal protein ingredients such as poultry-by-product meal. To date, each of these protein products, such as canola meal, soybean meal, and poultry-by-product meal have been processed (produced) separately and then these protein sources have been blended together in dried and finely ground form in appropriate ratios for a particular aquatic species at the time of diet formulation and preparation.

Most research on oilseeds has focussed on the use of products derived from processing soybeans, rapeseed/canola, sunflower seed or cottonseed. Comparatively few of these studies, however, have been directed to assessing the feasibility of using canola, flax seed, mustard seed, hemp and the like. Indeed, in the case of canola for example, although proteins contained therein are rich in lysine and methionine, both of which are limiting amino acids in most cereal and oilseed proteins, its use as a protein source in food products has been severely limited, due to the fact that the proteinaceous material which is left over after oil is extracted by known processes contains antinutritional constituents. The latter include insoluble and soluble fibres, glucosinolates (antithyroid compounds), phenolic compounds and phytic acid.

It has been shown that the concentrations of the above mentioned unwanted constituents should be minimized in order to allow full expression of the high quality of oilseed protein and to improve the overall digestibility, palatability, as well as bioavailability of minerals in the oilseed protein product. This is highly desirable when feeding either terrestrial species or aquatic species.

U.S. Patent No. 4,418,086 to Marino et al. discloses the preparation of an animal feed

which comprises (a) a proteinaceous matrix, (b) fat or oil, (c) a sulfur source, (d) farinaceous material, (e) a plasticizer and (f) water. The method disclosed involves the blending of the ingredients together, introducing the mixture into an extruder and subjecting it to shear forces, mechanical work, heat and pressure such that the product temperature prior to discharge is at least 280 degrees F. This patent is concerned with the production of an animal feed with a "meat like texture".

U.S. Patent No. 3,952,115 to Damico et al. relates to a feed where an amino acid is utilized as an additive to fortify a proteinaceous feed.

U.S. Patent No. 4,973,490 to Holmes discloses the production of animal feed products utilizing rape seed in combination with another plant species.

U.S. Patent No. 5,773,051 to Kim relates to a process for manufacturing a fish feed which refloats after initially sinking. This document discloses a process including blending conventional fish feed containing fish meal, wheat meal, soybean meal and other substances and compressing the mixture at a constant temperature to produce a molded product.

Furthermore, U.S. Patents No. 4,233,210 to Koch and No. 4,889,921 to Diosady et al. disclose preparations of protein concentrates for use in animal or human nourishment, from oilseeds including rapeseed (canola). The various processes of these inventions generally comprise heating, drying and distillation steps, as well as treatments with alkaline solutions and extractions with organic solvents.

The protein extract claimed by Cameron et al. in U.S. Patents Nos. 4,418,013 and 4,366,097; and by Murray et al. in U.S. Patents Nos. 5,844,086 and 6,005,076 is said to be "protein isolate", which is regarded as being different from a protein concentrate. Indeed, it is established that a protein extract is an isolate when the protein content exceeds 90% and the protein is undenatured. Accordingly, the process of the preparation of an isolate does not allow for a heating step at elevated temperature.

Lawhon et al. in U.S. Patent No. 5,086,166 disclose a process allowing for the simultaneous preparation of protein as precipitate or curd, and oil for use as food products or food ingredients, from numerous oilseeds including soybeans, glandless, cottonseeds, sunflower seeds, peanuts and sesame seeds. At an early step of the process, a heating treatment (at about 60°C to 90°C) of the material in water is performed, in order to inactivate enzymes inherent in the seed.

A process for the preparation of rapeseed and canola protein concentrates known as the "FRI-71 process" has been described by Jones (J. Amer. Oil Chem. Soc. 56, 1979, 716-721). This process allows for the production of highly digestible protein concentrates with reduced levels of antinutritional factors (except for phytic acid) that can be used to entirely replace the fish meal portion of diets for trout. However, subsequent work conducted in collaboration with the POS Pilot Plant Corporation in Saskatoon revealed that the FRI-71 process was not cost effective, due to low yields of the concentrates, and insufficient numbers of other value-added products apart from canola oil stemming from the process. Also, the process as described could not easily be applied in the private sector using existing oilseed and fish meal processing technology.

In the present invention, a modified FRI-71 process is described that results, besides the high value canola protein concentrate and animal feed grade canola oil, in other value-added products such as canola oil suitable for the organic food market, nutritionally upgraded canola meal, and products suitable as components in organic or predominately organic fertilizers. The process of the invention is simple and economical. Moreover, the process is readily integrated into existing oilseed crushing plants or fish meal production plants.

The process described in an embodiment of the present invention is further extended to various oilseeds including canola, rapeseed, sunflower seed, flax seed, mustard seed, cottonseed, hemp and soybeans. Moreover, mixtures of different oilseeds are also used in the process.

An object of the invention for certain embodiments is to provide an improved process for

extracting protein and oil (human and animal feed grade) from oilseed. A further object of other embodiments is to provide protein products that are particularly well suited for use in high energy (lipid) diets for fish farming and in some animal feeds.

### **Summary of the Invention**

In the present invention, there are several different aspects represented by different process aspects, as well as several novel product compositions resulting from different process aspects.

Dealing initially with the process aspects, there is provided a first aspect involving the preparation of nutritionally upgraded oilseed meals, which are protein and lipid-rich and have a reduced fibre content, and plant oils from oilseeds for use in fish or other non-human animal diets or human foods. This process comprises the steps of:

- providing a source of oilseed;
- subjecting said oilseed to heat treatment to substantially reduce the concentration of at least some antinutritional components normally present in said oilseed to obtain heat-treated seed;
- dehulling said heat-treated seed to produce a meat fraction and a hull fraction or a mixture thereof; and
- cold pressing said meat fraction or said mixture to yield said plant oils and said protein and lipid-rich meals.

20 According to a second aspect of the present invention, there is provided a process for preparation of nutritionally upgraded oilseed meals, which are protein and lipid-rich and have a reduced fibre content, and plant oils from oilseeds for use in fish or other non-human animal diets or human foods comprising the steps of:

- providing a source of oilseed;
- subjecting said oilseed to heat treatment to substantially reduce the concentration of at least some antinutritional components normally present in said oilseed to obtain heat-treated seed;
- providing a source of unhydrolyzed animal offal;
- blending said heat-treated seed in particulate form with said animal offal, and if

required water together with an antioxidant, to form a mixture thereof;

- cooking said mixture under conditions selected to substantially improve protein digestibility, and substantially free cellular water present in said animal offal, and if required as well as to facilitate separation of protein from the lipid in said oilseeds to obtain a cooked mixture; and
- separating said cooked mixture into a stickwater fraction, a moisture containing protein-rich fraction, and an animal feed grade oil fraction.

In a third aspect of this invention, the above-described second aspect can be modified as described herein to provide the third process aspect. In particular, in the above second aspect, the modifications involve the preparation of protein concentrates and lipid sources from co-processing of animal offal with oilseed for use in fish or other non-human animal feeds, wherein the cold pressing step of said meat fraction or said mixture obtained from the first aspect above is carried out so as to substantially reduce the particle size of said meat or said mixture and to yield a high value human grade oil and a protein and lipid-rich meal with reduced fibre content. Thus, the third aspect of the process comprises the further steps of:

- providing a source of unhydrolyzed animal offal;
- blending said protein and lipid-rich meal with said animal offal, and if required together with an antioxidant to form a mixture thereof;
- cooking said mixture under conditions selected to substantially improve protein digestibility, and substantially free cellular water present in said animal offal, as well as to facilitate separation of protein from the lipid in said animal offal and said oilseeds to obtain a cooked mixture; and
- separating said cooked mixture into a stickwater fraction, a moisture containing protein-rich fraction, and an animal feed grade oil fraction.

A fourth aspect of the process of the present invention involves the preparation of protein concentrates and lipid sources from the co-processing of animal offal with raw oilseeds for use in fish or other non-human animal diets. The fourth process aspect comprises the steps of:

- providing a source of oilseed;

- cold pressing said oilseed under conditions to substantially reduce the particle size of said oilseed and obtain pressed raw seeds;
- providing a source of unhydrolyzed animal offal;
- blending said pressed raw seeds with said animal offal, and if required water together with an antioxidant to produce a mixture thereof;
- cooking said mixture under conditions to substantially improve protein digestibility, and substantially free cellular water present in said animal offal and facilitate separation of protein from the lipid in said animal offal and said oilseed to obtain a cooked mixture; and
- separating said cooked mixture into a stickwater fraction, a moisture containing protein-rich fraction, and an animal feed grade oil fraction.

Desirably, in the above aspect of the present invention, there is provided a process for preparation of protein concentrates and lipid sources from the co-processing of animal offal with dried and then dehulled oilseeds for use in fish or other non-human animal diets. In this fifth aspect, the process further comprises the steps of:

- providing a source of oilseed;
- drying said oilseed to produce a dried seed;
- dehulling said dried seed to produce a meat fraction and a hull fraction or a mixture thereof;
- cold pressing said meat fraction or mixture under conditions selected to substantially reduce particle size of said meat or mixture to yield a high value human grade oil and protein and lipid-rich meals with reduced fibre content;
- providing a source of unhydrolyzed animal offal;
- blending said protein and lipid-rich meal with said animal offal to form a mixture thereof;
- cooking said mixture under conditions selected to substantially improve protein digestibility, substantially free cellular water present in said animal offal and facilitate separation of protein from the lipid in said animal offal and said oilseeds to obtain a cooked mixture; and
- separating said cooked mixture into a stickwater fraction, a moisture containing protein-rich fraction, and an animal feed grade oil fraction.

In a fifth aspect of the present invention, there is provided a process for producing a protein concentrate for use in animal and aquafeeds. As such, the fifth aspect process steps comprise:

- providing a source of oilseed ;
- drying said oilseed to reduce its moisture content to below about 10% to obtain dried seed or subjecting said oilseed to heat treatment under conditions selected to substantially deactivate, destroy or reduce the concentration of at least some of the antinutritional components normally present in the oilseed to produce a heat-treated seed;
- cold pressing or grinding said dried seed or heat-treated seed to reduce the particle size and yield human grade oil;
- providing a source of unhydrolyzed animal offal;
- blending said oilseed and said animal offal in ratios from about 10:90 to about 90:10 form a mixture thereof;
- cooking said mixture to obtain a cooked mixture prior to said extracting step;
- separating said cooked mixture into a stickwater fraction, a moisture containing protein-rich fraction, and an animal feed grade oil fraction;
- extracting said mixture with a solvent; and
- removing said solvent to obtain a protein concentrate.

In the first aspect of the process, as an optional feature, the process may further include the step of extracting said protein and lipid-rich meals with a solvent, and the step of stabilizing said plant oils by adding an antioxidant.

Further, there may also be included the step of drying the protein-rich fraction to reduce its moisture content to below about 10%. Moreover, the moisture content can be between 6% to 9%.

In a sixth aspect of the present invention, there is provided a process for preparation of oilseed protein concentrates from oilseed for use in fish or other non-human animal diets comprising the steps of:



- subjecting the oilseed to heat treatment under conditions selected to substantially deactivate, destroy or reduce the concentration of at least some of the antinutritional components normally present in oilseed to produce heat-treated seed;
- dehulling the heat-treated seed to produce a meat fraction and a hull fraction;
- cold pressing the meat fraction to yield a high value human grade oil and a moisture containing protein and lipid-rich meal having a reduced fibre content;
- blending the protein and lipid-rich meal with water and an antioxidant to produce a blended mixture;
- cooking the blended mixture under conditions selected to substantially improve protein digestibility to obtain a cooked mixture; and
- separating the cooked mixture into a stick water fraction, a moisture containing protein-rich fraction, and an oil fraction.

Further, in the above aspect of the process, the process may optionally include the steps of subjecting the protein and lipid-rich meal to enzymatic pH adjusted water treatment under conditions selected to substantially decrease the phytic acid concentration normally present in oilseed to thereby produce a protein and lipid-rich meal having reduced phytic acid and fibre contents.

Moreover, in the above embodiment, a further step of effecting a delay prior to subjecting the blended mixture to the cooking step.

A seventh aspect of the present invention provides a process for preparation of oilseed protein concentrates from oilseed for use in fish or other non-human animal diets comprising the steps of:

- drying the oilseed to produce dried seed;
- dehulling the dried seed to produce a meat fraction and a hull fraction;
- cold pressing the meat fraction to yield a high value human grade oil and a moisture containing protein and lipid-rich meal having a reduced fibre content;
- blending the protein and lipid-rich meal with water and an antioxidant to produce a blended mixture;

- cooking the blended mixture under conditions selected to substantially improve protein digestibility to obtain a cooked mixture; and
- separating the cooked mixture into a stick water fraction, a moisture containing protein-rich fraction, and an oil fraction.

Optionally, a step involving the initial sterilizing of the oilseed may be performed and the sterilization step can be performed using infrared energy.

There may be also included the step of cooking said mixture to obtain a cooked mixture prior to said extracting step. In this embodiment, there may be further included the step of separating said cooked mixture into a stickwater fraction, a moisture containing protein-rich fraction, and an animal feed grade oil. If desired, there also may be provided the step of drying the protein concentrate.

In another preferred embodiment, in the second process aspects, desirably the heat treatment is a rapid heat treatment. The heat treatment may be carried out in one or more stages - for example, a two stage heat treatment can be employed where temperatures range from about 100°C to 115°C, and for treatment times ranging from 1.5 minutes to 30 minutes or more depending on the specific components being treated.

Particularly suitable for the second process aspect, is where the oilseed is selected from the group consisting of canola, rape seed, soybeans, sunflower seed, flax seed, mustard seed, cotton seed, hemp and mixtures thereof. In the first process aspects, the oilseed may be selected from the group consisting of canola, sunflower seed, flax seed, mustard seed, and mixtures thereof. In the event the oilseed is a commercially available processed ground oilseed meal, the initial steps involving rapid heat-treatment and cold pressing are deleted.

In the second aspect of the invention, the animal offal may be selected from the group consisting of fish processing waste, whole fish, fish by-catch, squid offal, whole birds without feathers, beef offal, lamb offal and mixtures thereof. Particularly suitable in the sixth aspect is where the animal offal is a fish product or poultry or tail-end dehulled meal

(fibre-reduced). For instance, squid offal, poultry offal without feet, and whole birds including chickens, turkeys and others without feathers can be used. The fish offal or whole fish utilized include fish species having low levels of chlorinated hydrocarbons and heavy metals such as mercury. The animal offal can be a minced unhydrolyzed animal offal. The process may also include the step of dehulling the heat-treated seed and the blending step may include adding hot water to the mixture.

The dehulling step may be carried out by a mechanical treatment with a gravity screening or air-classification step and may also further include a seed sizing step. Optionally the oilseed can be treated by suitable techniques to remove the outer mucilage layer of the seed coat before the seed is used; the oilseed used includes flax seed. Especially when producing aquatic feeds, oilseed can be selected from canola, soybeans, sunflower seed, hemp or delinted cotton seed or mixtures thereof is used, due to their global availability, cost, and/or high quality of protein and/or lipid.

The cooking step may be performed at a temperature of from about 90°C to about 93°C and may further include the step of adding an antioxidant and/or a palatability enhancer to the cooked mixture. The antioxidant can be selected from the group consisting of ethoxyquin (santoquin), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone, natural antioxidants and mixtures thereof. One or more of the foregoing antioxidants are also added to the dried protein concentrate, and the animal feed grade lipid fraction. In the case of the former, the amount of antioxidant utilized is from about 200 ppm to about 250 ppm whereas the latter is supplemented with about 250 ppm to about 500 ppm antioxidant(s). Combinations of BHA and ethoxyquin or ethoxyquin alone at highest level is used. The palatability enhancer may be selected from the group consisting of natural and synthetic products based on krill, euphausiids and derivatives thereof, squid, Finnstim™ and mixtures thereof. Other ingredients such as enzymes, fillers, as well as other sources of lipid of plant or animal origin and other protein sources such as heat-treated field peas or lupins may be added to the composition of the mixture.

The oilseed and the animal offal in the second process aspect is mixed together in a ratio

of about 10:90 to about 90:10 by weight. It can be seen that the mixed ratio can be from about 25:75 to about 75:25 by weight or from about 60:40 to about 40:60 by weight.

The amount of oilseed present in the mixture depends upon the sources of oilseed and animal offal actually used. This amount also depends on respective attendant concentrations of protein and lipid, as well as costs. For instance, the oilseed can be present in a range of about 5% to about 78% by weight. More particularly, the oilseed can be present in the amount of about 22% to about 78% by weight, or the range of about 40% to about 60% by weight. It is important to maintain an optimal ratio of water (from endogenous and exogenous sources) to the oil-free dry matter content of the oilseed in the initial mixture and usually this is found within the range of about 3-6:1 w/w. Ratios within this range facilitate the removal of water soluble antinutritional factors from the oilseed (in press liquor).

The mixture is further pressed and/or centrifuged using respectively either a screw press equipped with perforated screens, an expeller equipped with flat steel bars set edgewise around the periphery and spaced to allow the fluids to flow between the bars, a decanter centrifuge or any combination of these. Depending upon the efficiency of liquid/solid separation the mixture is centrifuged before or after the presscake has passed through the screw press or expeller. This part of the process removes fluids generally comprised of water that contains some soluble protein and water soluble antinutritional factors stemming from the oilseed such as glucosinolates, phenolic compounds and unwanted sugars including oligosaccharides (raffinose and stachyose). Animal feed grade plant oil that is enriched with fatty acids from the animal offal lipid is also removed.

The drying step in the second to sixth process aspects may be performed at a temperature of between about 70°C to about 85°C. As mentioned above, the separation step may be carried out in a screw press, expeller press or decanter centrifuge, or any combination thereof. As an optional feature, the stickwater fraction obtained after separation may be further condensed to yield condensed solubles. The step of stabilizing the condensed solubles can be with an inorganic acid.

The step of incubating the mixture in the presence of one or more enzymes prior to the cooking step may further be included. An enzyme which can be used includes the enzyme phytase.

When a palatability enhancer is utilized, it may be selected from conventional products based on krill, euphausiids, and/or squid or other like palatability enhancers such as Finnstim™ or the like. The palatability enhancers may be added to the dried protein concentrates in amounts ranging from about 1% to about 3% by weight.

The cooking step is carried out using a heat exchanger or through direct steam injection coupled with batch processor. The process may further comprise, if desired, the initial step of deboning the animal offal to produce deboned animal offal and bones.

The cold pressing step should be carried out at a temperature not exceeding 85°C, desirably below about 70°C.

The source of the oil seed utilized is most desirably a commercially available particulate processed oil seed meal, which has not been previously subjected to initial rapid heat treatment or cold processing.

The extraction step may be carried out at least twice; the solvent that can be used is includes hexane.

The processes which involve processing of oilseed prior to co-processing it with animal offal, can utilize the addition of hot water (from about 37°C to about 55°C) to ground oilseed, followed by adjustment of the pH to a value of from about 5.5 to about 6.0 using an inorganic acid such as sulphuric acid; this treatment being carried out in the presence of an enzyme such as the enzyme phytase.

The various processes of the present invention can be economically and readily carried out using conventional equipment. Such processes will provide cost effective products which can be used in place of or added to other known products in order to achieve

additional sources of the desired ingredients for use in fish or other non-human animal diets or human foods. The use of inexpensive fish wastes and other animal offal in the various processes of the present invention is a positive way to deal with waste streams rather than considering them as a liability.

As described hereinafter, it will be seen that the different processes can be combined into one overall procedure allowing separation of products at various stages of the process.

Turning now to the various novel products and compositions according to the invention, the first product relates to a protein source for use in animal and aquafeeds comprising an animal product selected from animal offal, whole fish too small for filleting operations, fish by-catch and whole birds with or without feathers in an amount of about 22% to about 90% by weight; and

- oilseed in an amount of about 10% to about 78% by weight; and
- wherein the animal product and the oilseed have been co-processed and cooked together, following which fluids have been removed therefrom to obtain a pressed mixture which has been dried to provide the protein source.

According to the above first, the product aspect relates to a protein source having from about 40% to about 80% protein, desirably from about 55% to about 77% protein calculated on a lipid-free dry weight basis, said source being adapted for use in animal and aquafeeds and comprising an admixture of treated oilseed protein and animal offal whereby said admixture is characterized by at least one of the following:

- enriched concentrations of essential amino acids and bioavailable minerals relative to those present in said animal offal or untreated oilseed;
- enriched concentrations of highly unsaturated n-3 fatty acids relative to those present initially in said oilseed if said source of animal offal is fish;
- reduced concentrations of heat-labile and water soluble antinutritional factors in an amount of at least 20% by weight relative to non-treated oilseed protein;
- increased protein digestibility relative to non-treated oilseed protein; and
- a lipid concentration of less than 10% of dry weight of said source.

In the first product aspect, the above product aspect of the invention has a reduced content of heat-labile and antinutritional components of at least 80% calculated on a lipid-free dry weight basis.

An antioxidant can be included which is selected from the group consisting of ethoxyquin (santoquin), butylated hydroxyanisole, butylated hydroxytoluene, tertiary butyl hydroxyquinone, natural antioxidants and mixtures thereof. The amount of antioxidant utilized will range depending on the components; generally speaking, this will be from about 200 ppm to about 250 ppm in the protein concentrate, and the animal feed grade lipid fraction resulting from the production of the concentrate may be supplemented with about 250 ppm to about 500 ppm antioxidants. A combination of BHA and ethoxyquin or ethoxyquin alone at its highest levels can be used.

Also, the product can include an enrichment of at least one amino acid which can be selected from the group consisting of arginine, histidine, isoleucine, leucine, lysine, methionine, cystine, phenylalanine, tyrosine, threonine, tryptophan, and valine. Preferred amino acids altered in this product are selected from lysine, methionine or cystine. Further, an enrichment of at least one mineral can be selected from the group consisting of calcium, phosphorus, magnesium, sodium, potassium, copper and zinc. Preferred minerals altered can be selected from calcium, phosphorus, sodium, zinc or mixtures thereof.

20 This product can comprise an enrichment of at least one n-3 highly saturated fatty acid; this is preferably at least one fatty acid selected from eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) if the source of animal offal is fish.

The heat-labile and water soluble antinutritional components can be selected from glucosinates, phenolic compounds including sinapine, chlorogenic acid, oligosaccharides, trypsin inhibitor, saponins and isoflavones or mixtures thereof.

The digestibility of a product of the invention can be about at least 89% for Atlantic salmon in sea water (fecal settling columns or the Guelph System of fecal collection was

used). This percentage may vary and is desirably as high as possible, e.g., in the order of from about 92% to about 100%.

The oilseed in the first product of the invention is selected from the group consisting of canola, rapeseed, soybeans, sunflower seed, flax seed, mustard seed, cotton seed, hemp and mixtures thereof. In preferred embodiments, as an optional feature, the oilseed may be heat-treated.

The animal offal in the first product is most desirably selected from the group consisting of whole fish, fish by-catch, fish processing waste, squid offal, whole birds without feathers, beef offal, poultry offal, lamb offal and mixtures thereof.

The protein and lipid contents of the first product are present in an amount within the range (respectively) of about 50% to about 77% calculated on a lipid-free dry weight basis and less than about 10% by weight if the step involving organic solvent extraction has been employed.

Phytate-reduced protein concentrates can be produced. The process involves an additional step consisting of adding hot water (temperature of about 37°C to about 55°C) to the ground oilseed in the presence of the enzyme phytase. It should be mentioned that the moisture content of the ground oilseed should be raised to about 80% or more and the pH should be about 5.5 to about 6.0 by addition of an inorganic acid, such as sulphuric acid. The mixture is then incubated for about 30 minutes and not more than 240 minutes, before being mixed with the animal offal.

The protein source finds particular use for animal and fish feeds to cost effectively and extensively replace high nutritive value protein sources such as premium quality fish meal, or conventionally processed oilseed meals that have lower nutritional value. The advantage of the above products according to the present invention, is that they may be produced in a very economical manner by co-processing sources of protein that heretofore have been processed separately without the attendant benefits of enhancing the nutritive value of the oilseed protein fraction through protein and mineral



complementation from the animal offal and by concurrent reduction of the concentrations of heat-labile and water soluble antinutritional factors as well as phytic acid if the optional initial step of phytase pretreatment of the oilseed is adopted. These protein products provide significant advantages to animal and fish feed manufacturers which in addition to the economic savings, also provide highly desirable and digestible proteins that have excellent amino acid profiles relative to the essential amino acid needs of commercially important animals and fish.

A second product aspect of the invention relates to another product which is an edible organic oil comprising an oilseed oil, said organic oil having been obtained by cold pressing oilseed in which the cold pressing was carried out at temperatures below 85°C, said oil having minimal lipid oxidation products and a peroxide value of less than about 2 milliequivalents per kg following oilseed processing.

The oilseed providing the oil of the second product is preferably selected from canola, rape seed, sunflower seed, flax seed, mustard seed, cotton seed and mixtures thereof. Optionally, the oilseed can be heat-treated.

The edible organic oils of this aspect of the present invention provide highly nutritional products which can be used for human consumption. Such oils may be packaged and distributed per se or may be incorporated into various types of foods or food compositions where edible oils are required or utilized. A further advantage of such organic oils is that they have not been subjected to any organic solvent or other processing steps that would reduce their concentrations of natural antioxidants. Moreover, they are generated under conditions that minimize lipid peroxidation and the products that result from the process. They are highly desired by health conscious people who are concerned with ingesting vegetable oils close to their natural state. Hence, these oils command a premium price in the market place.

A third product aspect relates to an animal feed grade oil for use in animal and aquafeeds comprising an admixture of treated oilseed oil and animal offal, said admixture having an enriched n-3 highly unsaturated fatty acid content (20:5n-3 + 22:6n-3) relative

to non-treated oilseed oil if the animal offal used is fish or poultry that have been fed diets comprising adequate concentrations of one or more fish products. Preferred oilseeds in this embodiment is oil derived from canola seed since the product may further comprise an enriched monounsaturated fatty acid content (18:1 n-9) relative to non-treated oilseed oil.

The feed grade oils will find utility in animal and fish foods; they have the advantage that they can be produced in a very efficient and economic manner and they provide highly nutritional sources of enriched unsaturated fatty acid contents. The latter lipid sources are highly desirable particularly for use in fish feeds to partially replace premium quality fish oil that may be expensive and difficult to obtain. This is specially true if the plant oil fraction has been enriched with n-3 highly unsaturated fatty acids from the fish offal fraction. These oils can be utilized individually or, if desired, combined with other known and conventional oils at the time of feed manufacture.

A fourth product aspect relates to a constituent for an organic fertilizer comprising at least one of canola, sunflower, soybean, mustard seed, cotton seed and hemp hulls, said hulls being dried hulls and containing protein and lipid. The hulls are heat-treated hulls. As a constituent for organic fertilizers, products can be used in combination with other conventional fertilizer components such as sawdust. As a result of adding this constituent, fertilizers have the advantage of a readily available source of nitrogen. The hulls will act as soil conditioner and carrier for nutrients, these being delivered to the soil on a sustained basis. In addition, the fourth product of the invention will facilitate soil irrigation and water retention in soils. This feature is particularly important in times of drought.

A fifth product aspect relates to a composition of condensed solubles for use as constituents in organic fertilizers comprising an admixture of treated oilseed and animal offal whereby said admixture has an enriched soluble nitrogen content, water soluble carbohydrate content, water soluble antinutritional component content and mineral content.

The original hull fraction can be directed for use in ruminant diets either as is or pretreated with carbohydrases. The original hull fraction can be used in the production of organic fertilizers where it serves as a carrier medium that is completely broken down enzymatically during aerobic or anaerobic decomposition processes.

The oilseed in the fifth product is selected from canola, rape seed, soybeans, sunflower seed, flax seed, mustard seed, cotton seed, hemp and mixtures thereof. Optionally in this fifth product, the oilseed may be heat-treated.

The animal offal in the fifth product of the invention is selected from fish processing waste, whole fish, fish by-catch, squid offal, whole birds without feathers, beef offal, lamb offal and mixtures thereof.

The water soluble antinutritional component can be selected from glucosinates, phenolic compounds including sinapine, chlorogenic acid, oligosaccharides, saponins or isoflavones. Further, the soluble carbohydrate can be selected from monosaccharides, disaccharides and oligosaccharides, while the mineral can be selected from calcium, phosphorus, magnesium, sodium, potassium, copper, iron and zinc.

This product can be enriched with soluble nitrogen, phosphorus, potassium, as well as organic nutrients. As a constituent for organic fertilizers this product contributes to upgrade the quality of the fertilizer. It should stimulate plant growth, specially the root structure of plants.

- 20 The condensed soluble products can be utilized with other fertilizer components to provide enhanced fertilizers. Also relating to fertilizers, the condensed solubles can be incorporated into known fertilizers or, if desired, could be marketed as additives per se to known fertilizers.

There is also provided a sixth product according to the present invention which relates to a protein and lipid-rich oilseed meal suitable for use in fish and non-human animal diets. This product comprises a heat-treated dehulled oilseed, said oilseed being

substantially free of flaxseed, mustard seed, rapeseed and cotton seed, said meal having:

- from about 26% to about 40% protein on a dry weight basis;
- from about 48% to about 64% protein on a lipid-free dry weight basis;
- from about 2.4% to about 4.6% methionine and cystine calculated as a percent of said protein;
- from about 3.6% to about 6.1% lysine calculated as a percent of said protein;
- from about 21% to about 52% lipid on a dry weight basis;
- from about 2% to about 12% crude fibre on a lipid-free dry weight basis;
- from about 0.16% to about 0.45% calcium on a lipid-free dry weight basis; and
- less than about 0.01% sodium on a lipid-free dry weight basis.

The sixth product of the present invention may further comprise at least one of glucosinolates, sinapine, chlorogenic acid and mixtures thereof. In preferred embodiments of this sixth product aspect, the glucosinolates are in an amount of up to about 20  $\mu$ moles of total glucosinolates per gram on a lipid-free dry weight basis. Further, the protein is in an amount from about 30% to 33% and lipids are in an amount from about 30% to 38%.

A trypsin inhibitor can be included in an amount of up to about 8000 units/g on a lipid-free dry weight basis; sinapine can be included in an amount of up to about 2.1% on a lipid-free dry weight basis; and the chlorogenic acid can be in an amount of up to about 3 % on a lipid-free dry weight basis.

Optionally, the oilseed can be partially or totally dehulled.

Protein and lipid rich meals can be produced in a very economical manner and will find utility in fish and animal feeds requiring high protein and lipid rich meal with reduced concentrations of fibre and heat-labile antinutritional factors. . Their utility will depend on various factors such as the species of animal or fish and their respective requirements for protein and energy, etc. As described previously with respect to other animal and fish feed sources, the products of this aspect of the invention can be incorporated into the

feeds of animal and fish as replacements for conventionally processed oilseed meals and oils, and fish meals and oils. Due to the protein and lipid rich content of such products, a beneficial result will be obtained in the increased digestible energy content of diets for such animals and fish. The protein concentration can also be increased in the preceding meals through removal of lipid by solvent extraction which increases their utility as components in low energy diets for animals and fish.

In a seventh product aspect of the present invention, there is provided a protein concentrate containing an admixture of a co-processed oilseed and unhydrolyzed animal offal, said concentrate being suitable for use in fish and non-human animal diets, said oilseed comprising a heat-treated dehulled oilseed substantially free of flaxseed, mustard seed, rapeseed and cotton seed, said protein concentrate having:

- from about 38% to about 58% protein on a dry weight basis;
- from about 52% to about 77%, desirably up to about 57% protein on a lipid-free dry weight basis;
- from about 2.7% to about 4.6% methionine and cystine calculated as a percent of protein;
- from about 4.3% to about 7.9% lysine calculated as a percent of said protein;
- from about 24% to about 37% lipid on a dry weight basis;
- from about 1.7% to about 10% crude fibre on a lipid-free dry weight basis;
- from about 0.7% to about 3.6% calcium on a lipid-free dry weight basis; and
- from about 0.06% to about 0.30% sodium on a lipid-free dry weight basis.

The seventh product may further comprise at least one of glucosinolates, sinapine, chlorogenic acid and mixtures thereof. In preferred embodiments of this seventh product aspect, the glucosinolates are in an amount of up to 4.0  $\mu$ moles of total glucosinolates per gram on a lipid-free dry weight basis.

Moreover, in a preferred embodiment of the seventh aspect, the product aspect contains from about 50% to about 78% protein and from about 7% to about 12% lipid/

A trypsin inhibitor can be included in an amount of up to about 2500 units/g on a lipid-

free dry weight basis; sinapine can be in an amount of up to about 1.2% on a lipid-free dry weight basis; and the chlorogenic acid can be in an amount of up to about 1.7% on a lipid-free dry weight basis.

If desired, the oilseed can also be partially or totally dehulled.

The high digestible protein content, moderate content of highly digestible lipid, reduced fibre content and substantially reduced heat-labile and water soluble antinutritional factor content make them suitable as major replacements for fish meal and other conventional sources of protein used in fish and non-human animal diets. Their enriched content of at least some of the essential amino acids and minerals, together with their economical cost of production will make such products highly desirable as feed stuff commodities throughout the world.

In an eighth product aspect of the present invention, there is also provided an animal feed grade oil comprising oil derived from an admixture of a co-processed oilseed and unhydrolyzed animal offal, said oil being substantially free of flaxseed oil, mustard seed oil, rapeseed oil and cotton seed oil, said animal feed grade oil having:

- from about 60% to about 92% of total fatty acids as unsaturated fatty acids;
- from about 8% to about 50% of total fatty acids as (n-6) fatty acids;
- from about 0.5% to about 35% of total fatty acids as (n-3) fatty acids;
- from about 3% to about 25% of total fatty acids as n-3 highly unsaturated fatty acids; and
- a peroxide value less than about 8 milliequivalents per kg of oil at the time of production.

The oilseed can be a raw oilseed or a heat-treated oilseed. The animal offal can be a fish product and the product further comprises (20:5n-3+22:6n-3).

There is a generally high content of n-3 highly unsaturated fatty acids compared to the oil from the initial oilseed used if the source of animal offal is fish and hence it is desirable for use in both fish and animal diets. The additional benefits of this type of

product include ease of production, economical attributes, readily available sources of natural products for obtaining the oil, and its adaptability to incorporation into existing animal diets, as well as its utility as a separate dietary component.

A ninth product aspect of the present invention relates to an edible organic oil comprising oil of cold pressed heat-treated oilseed, said oil being substantially free of flaxseed oil, mustard seed oil, rapeseed oil and cotton seed oil, said organic oil comprising:

- from about 86% to about 96% of total fatty acids as unsaturated fatty acids;
- from about 20 to about 80% of total fatty acids as (n-6) fatty acids; and
- a peroxide value of less than about 2 milliequivalents of peroxide per kg oil at

the time of production.

The ninth product may further comprise up to about 22% of total fatty acids as (n-3) fatty acids.

The oilseed can be undehulled, partially dehulled or totally dehulled if desired. This product is a very cost-effective organic oil for the increasing organic human food industry and will find utility in various types of food products or as a separate product in and of itself. Depending on the intended utility, the animal feed grade oil can be an oil derived from raw oilseed or the edible organic oils may be derived from raw oilseed.

It will be understood that reference to the above described products which are suitable for animal and fish feeds, refers to products which can be used by numerous types of species. For example, depending on the geographic location, fish feeds are used in fish farming operations for salmon, trout, tilapia, carp, catfish, sea bream and many other warm water as well as cold water species of commercial importance. In the case of animal feeds, conventional farming practices utilize such feeds for poultry, hogs, swine and cattle.

In further explanation of the various embodiments of both the products and process aspects of the present invention, the solvent used for extracting the mixture obtained from co-processing of oilseed and animal offal includes hexane or other compatible

solvents used in the animal feed or human food industry.

In various embodiments of the process and product aspects of the present invention, the ash content in the protein concentrates can be regulated as desired by controlling the concentration of bone in the animal offal. Thus, the ash can be controlled by using a deboning step to obtain offal with the desired bone content. Bones in wet or dry form of different types of offal can be utilized, with varying degrees of bone coarseness. By way of example, the ash content can thus be controlled by controlling the amount of bone added to the mixture of oilseed and animal offal.

In the process and product aspects of the present invention, when referring to animal offal such as birds or chickens, it is to be understood that a most preferred embodiment is the use of offal without bird feathers.

In both the process and product aspects of the present invention, when using dehulled seeds, the term "dehulled" is intended to mean seeds which have substantially all of their hulls removed. However, in many cases, partially dehulled seeds can be employed as otherwise noted herein, and to this end, dehulled seeds are those which have had at least 55% of their hulls removed.

The above described products can be produced by the various processes described herein; specific embodiments of such processes producing the products will be described hereinafter in greater detail.

20 As used in the specification, the term "unhydrolyzed" in describing the animal offal refers to animal offal which has less than about 20% by weight of hydrolyzed content, desirably less than 5% and most desirably no hydrolysis whatsoever (fresh, unspoiled). In most preferred embodiments, the amount of hydrolyzed content is as close as possible to 0% in order to best achieve the highest nutritive value in the products that are formed.

In this invention, the animal offal is preferably in a particulate form such as that which



would be obtained by processing procedures resulting in minced offal. Well known techniques in the offal processing art can be employed to obtain such minced offal.

### **Brief Description of the Drawing**

Having generally described the invention, reference will be made to the accompanied drawing which illustrates the preferred embodiments only.

Figure 1 is a schematic representation of the process according to the present invention. Figure 2 is a schematic representation of the process according to a preferred embodiment of the present invention.

### **Detailed Description of the Invention**

The steps involved in the process of the invention are broadly represented in Figure 1. In this Figure, there is illustrated a schematic representation of the co-processing of animal offal(s) with oilseed(s) to yield cold pressed oil indicated as product 1; hulls from dehulled oilseed meats indicated as product 2; nutritionally upgraded oilseed meal produced from heat treated, dehulled and cold pressed oilseed indicated as product 3; animal-feed grade oil indicated as product 4; condensed solubles indicated as product 5; and high nutritive value protein concentrate indicated as product 6. Other products of the invention are obtained by further processing the above-mentioned products as will be described in greater detail hereinafter.

In accordance with certain embodiments of the invention, undehulled oilseed (A) is used in the process. Other embodiments involve dehulled seed (B) and raw seed. Dehulled seed is preferred when it is desired to feed monogastric species such as fish and poultry, and the preferred raw seed used in this embodiment includes canola, sunflower, or delinted cottonseed. Undehulled oilseed (A) or dehulled oilseed (B) for monogastric diets may be used.

With respect to Figure 2, the subsequent steps are provided for with respect to an optional lipid extraction and solvent recovery step can be inserted to reduce the lipid content of the oilseed meal and just before or after press cake drying to reduce the lipid

content of the protein concentrate.

The initial step involves cold pressing (temperatures <85°C using a suitable cold press) the raw whole oilseed or alternatively subjecting the raw seed to rapid heat treatment and then dehulling and cold pressing the heat-treated seed. If the latter option is selected, one procedure involves heating the seed at 110-115°C for 90 seconds followed by an additional heating at 100-110°C for 30 min. Other options require less heat depending upon the form of heat and whether or not a vacuum is applied during the heat process.

The temperature and length of the treatment is

selected to substantially: (i) deactivate or destroy the activity of enzymes such as myrosinase, which is the enzyme responsible for glucosinolate hydrolysis in canola; (ii) improve the digestibility or bioavailability of the carbohydrates present in canola and other oilseeds; and (iii) reduce the moisture content in the seed, which results in a partial separation of the meat from the fibrous indigestible hull.

The dehulling process is further completed by subjecting the heat-treated seed through an impact, a disc, or other mechanical process coupled with a gravity screening or air-classification process. Other techniques may also be used in the dehulling process, and some of these may include sonic techniques.

The oilseed meats resulting from the cold pressing of the raw, unheated seed are not marketed directly for use in high energy animal feeds, unlike those originating from the cold pressing of heated, dehulled seed. Indeed, the latter have been nutritionally upgraded due to their reduced content of fibre and one or more heat-labile antinutritional factors. This meal contains about 30-33% protein and 30-38% lipid. It may be used as is or it may be further subjected to solvent extraction involving hexane, with subsequent recovery of the solvent and the meal to reduce its lipid content, thus elevating its protein concentration. The meal may be directly channelled into diets of aquatic and terrestrial species, or similar to the meal from the unheated, pressed seed, submitted without lipid extraction to the next step of the process. The cold pressed oils from both sources, however, are channelled into the organic human food market.

The meals from undehulled or dehulled oilseed are blended with a suitable amount of water (about 4-8:1 w/w water to oil-free dry matter of meal) and an antioxidant (e.g. 100 mg of santolquin/kg of meal). The added water serves to wash the oilseed meal as the blend moves through the cooker to either a continuous screw press that is surrounded with perforated screens or an expeller press. As the presscake moves through this stage of the apparatus, fluids are drained off that include water that contains soluble protein, some of the remaining water soluble antinutritional components such as glucosinolates (when canola is used), phenolic compounds and unwanted sugars like raffinose and stachyose; as well as a large portion of the lipid fraction. The mechanical separation of the aforementioned solids and liquid fractions may also involve the use of a decanter centrifuge depending upon the efficiency of liquid/solid separation after the presscake has passed through the screw press or expeller.

Thereafter, the fluid mixture is separated by continuous centrifuge into stick water and animal feed grade oil fractions (the latter may be subjected to additional processing steps as referred to previously to create a human grade oil). The press-cake meal is dried using a low temperature process (temperature of about 60°C to about 83°C) to yield a dried protein-rich fraction (concentrate).

In cases where the lipid content of the dried protein fraction is too high for the desired animal feed use, a solvent extraction step involving hexane is performed, with subsequent recovery of the solvent and the animal feed grade oil. In another embodiment of the invention, the solvent extraction step is performed prior to the low temperature drying step.

The stick water fraction mentioned above is condensed to about a third of its original volume and following acid stabilization, is then used together with the hulls as components in organic fertilizers for agriculture.

The oilseed in accordance with the present invention is selected from canola, rape seed, soybeans, sunflower seed, flax seed, mustard seed, cotton seed and hemp or mixtures of these oilseeds. The oilseed used in the process of the invention can also consist of

a mixture of two or more different oilseeds selected from the above-mentioned oilseeds. A suitable selection of oilseeds to be mixed together in the process will provide for products with enhanced nutritional values. With respect to the protein product, the amino acid profile can be obtained through amino acid complementation. Further, oil could be upgraded through, for example using a mixture of oleic acid, sunflower or yellow mustard with flax seed and/or canola. Such oil would have high oleic acid content and low or intermediate concentrations of linolenic acid. Also, these oils will have reduced concentrations of linoleic acid.

In accordance with the invention, oilseeds having a high content of phytic acid, such as canola, sunflower and hemp can be subjected to enzymatic pH adjusted water treatment prior to being used in the process. This pretreatment involves adjustment of pH to about 5.0 to about 5.5 and addition of enzyme phytase. The oilseeds in particulate form are incubated with phytase for about 4 hours or more, at a temperature of about 50°C to about 55°C. In the case of hemp, the seeds have to undergo a sterilization step, to prevent germination and this may be accomplished by using infrared energy or other suitable techniques. The dehulling step is imperative in the case of flax seed. Alternatively, removal of at least the mucilage layer in the outer seed coat should be carried out.

When sunflower or canola is used in the process of the invention in the production of protein concentrates, the heat treatment step may be avoided, however, in order to facilitate dehulling (specifically if mechanical dehulling is being performed), the seeds are subjected to a drying step, to reduce their moisture content to about 5%.

Given the above teachings, it will be seen that the invention also provides protein concentrates produced by the above process, containing from about 50% to about 78% protein, that are highly digestible and significantly depleted in antinutritional constituents (except for phytic acid in some cases if the seeds are not pretreated with phytase) that were present in the original oilseed. The oilseed protein concentrates of the present invention have moderate contents of lipids (from about 5% to about 12%) that include highly digestible monounsaturated and polyunsaturated fatty acids. The following

examples are presented to describe embodiments of the invention and are not meant to limit the invention unless otherwise stated.

Examples 1 to 10 outlined below described each step involved in the process of the invention:

#### EXAMPLE 1: Animal offal

A common batch of whole Pacific herring was used as the main source of animal offal for the project. Soon after the herring were caught, they were rapidly block frozen by McMillan J.S. Fisheries Ltd., Vancouver, BC and stored at -40°C for about 9 months. At this time, about 500 kg of herring were transported to the Department of Fisheries and Oceans, West Vancouver Laboratory where they were held at -20°C until small batches of about 50 kg were partially thawed for each test run. The thawed herring were cold extruded using a Butcher Boy equipped with an auger, cutter knife, and perforated plate having holes with diameter 9.52 mm.

Fresh poultry offal (heads and viscera minus feet) was also used for some trials that involved co-processing the offal with partially dehulled animal feed grade sunflower seed (designated as batch 2 hereinafter). The offal was obtained from West Coast Reduction Ltd., Vancouver, BC and was stored for one night at -20°C under cover before being handled as described above for the herring.

#### EXAMPLE 2: Oilseeds

The four oilseeds that have been tested successfully in this project include Goliath canola seed (Cloutier Agra Seeds Inc., Winnipeg, MB), soybeans (InfraReady Products Ltd., Saskatoon SK), sunflower (completely dehulled confectionary grade seed obtained from North West Grain, St. Hilaire, MN, USA (batch 1) and unde-hulled animal feed grade seed obtained from Cargill Incorporated, Wayzata, MN, USA; batch 2), and devitalized hemp seed (Seedtec/Terramax, Qu'Appelle, SK sterilized by InfraReady Products Ltd., Saskatoon SK). Delinted glandless cottonseed (California Planting Cottonseed Distributor, Bakersfield, CA, USA) and brown flax (InfraReady Products Ltd., Saskatoon, SK) were also tested in the process. The analytical results pertaining to products based

on the former are pending. It was concluded that flax seed would be suitable for the process provided that the seed is almost totally dehulled or the outer mucilage layer of the seed coat is removed through an economical process.

#### EXAMPLE 3: Heat treatment or micronization of oilseeds

In a preferred embodiment of the invention, specially for canola, soya, flax and hemp, an initial heat treatment was performed. The process involved subjecting the whole seeds to infrared energy so that the seed temperature reached 110-115°C for 90 seconds. Subsequently, the micronized seeds were held for 20-30 min, depending upon the seed source, in an insulated tank where temperatures ranged from 100-110°C (residual cooking conditions). These conditions inactivated enzymes such as myrosinase in canola and trypsin inhibitors in soya as well as peroxidase and cyanogenic glucosides. Further, they ensured devitalization of viable germ tissue in hemp, improved starch digestibility, and destroyed or reduced the concentrations of heat labile antinutritional factors other than those mentioned above.

Sunflower seeds (batches 1 and 2) were not micronized before co-processing with animal offal but the batch 1 seeds were dried to £ 10% moisture to ensure proper seed storage and facilitate dehulling. Thus, only non-micronized dehulled sunflower seeds were tested in this study.

#### EXAMPLE 4: Oilseed dehulling

Micronized canola, soya, hemp and flax and non-micronized animal feed-grade sunflower were dehulled. The process involved seed sizing, impact dehulling (Forsberg model 15-D impact huller), screening and air classification (Forsberg model 4800-18 screener and screen-aire).

#### EXAMPLE 5: Oilseed cold-pressing

In a preferred embodiment of the invention, the oilseeds (micronized or raw), except soya and micronized dehulled hemp were cold-pressed at a temperature not exceeding 85°C, using a Canadian designed and manufactured laboratory scale Gusta cold press (1 HP

Model 11, Gusta Cold Press, St. Andrews, Manitoba, Canada). This served to remove some (dehulled seeds) or a significant proportion (undehulled seeds) of the residual oil (organic human food grade oil) and concomitantly reduced the particle size of the oilseed before it was co-processed with minced animal offal in various proportions (improved the efficiency of the subsequent aqueous extraction of the water soluble antinutritional factors and oligosaccharides present in the oilseed).

In a more preferred embodiment, specially for soya, the particle size was further reduced, using a modified crumbler (model 706S, W.W. Grinder Corp., Wichita, Kansas). This machine was equipped after modification with dual motorized corrugated rolls. One of these had a fixed speed whereas the speed of the other could be varied. For the purpose of this investigation, the variable speed roller was adjusted to rotate much faster than the fixed speed roller to achieve a shearing action.

#### EXAMPLE 6: Mixing or co-processing step

Thawed, ground, whole animal offal (mostly herring, but in two cases poultry offal minus feet, was used) and oilseeds that had been micronized or dried as described in Example 3 or in raw form and either cold pressed or ground as described in Example 5 were first combined in various proportions. In preferred embodiments, the usual percentages of offal to oilseed were 75:25; 50:50; or 25:75 (w/w). Thereafter, 100 mg of santoquin (antioxidant) per kg of mixture in a marine oil carrier (1g/kg) were added. Then hot water was added to the mixture in such a way that the ratio of water to oil-free dry matter present in the oilseed was maintained between 3-6:1 (w/w), depending upon the source and proportion of oilseed in the mixture. Both the endogenous water originating from the offal and the exogenous water were considered when calculating the aforementioned ratios.

#### EXAMPLE 7: Cooking step

The mixture obtained from co-processing of animal offal and oilseed (Example 6) was cooked for about 27 min at 90-93°C in the steam jacketed cooker section of a pilot-scale fish meal machine (Chemical Research Organization, Esbjerg, Denmark), that was

equipped with a heated auger (it is noteworthy that the cooking step could have also been performed by using a heat exchanger with a positive displacement pump or through direct steam injection coupled with processor). The cooking step was undertaken to: (1) minimize the loss of soluble protein through protein denaturation, (2) destroy or reduce the concentration of heat labile antinutritional factors present in the oilseed (especially important when processing non-micronized seeds and micronized soya), (3) liberate the bound cellular water and lipid in the offal and the oilseed, and (4) subject the oilseed to aqueous washing to facilitate removal of the water soluble antinutritional factors originating from this source.

#### EXAMPLE 8: Pressing step

Significant but not total removal of the latter as well as lipid (animal-feed grade product) was accomplished by passing the cooked mixture through the fish meal machine screw press that was equipped with perforated screens and then a laboratory-scale press (Vincent model CP-4; Vincent Corp., Tampa Florida). Constituents in the water fraction of the press liquids consisted of water soluble carbohydrates such as monosaccharides, disaccharides, or problem sugars like raffinose and stachyose, phenolic compounds, glucosinolates (when canola used), chlorogenic acid (when sunflower used), isoflavones and saponins (when soybeans used) as well as some soluble nitrogen and water soluble vitamins. In preferred embodiments, the presscake in each case was dried in the steam jacketed drier portion of the above-mentioned fish meal machine at 75-83°C to produce dried protein and lipid-rich products.

#### EXAMPLE 9: Drying step

In one preferred embodiment, further drying of the protein products was necessary to reduce their moisture content. The drying was performed for about 30 min to reduce their moisture content to less than 10%. This was accomplished using a custom designed vertical stack (stainless steel mesh trays) pellet cooler that was equipped with two electric base heaters and a top mounted variable speed fan. The temperature of the upward drawn air was maintained between 70°C and 80°C during the process. All protein and lipid sources stemming from the above process, including the cold-pressed



oils were further stabilized with santoquin (ethoxyquin). In a more preferred embodiment, specially in the case of the dried protein products, 100 mg of santoquin were added per kg of product in a marine oil carrier (1g/kg). Then, each of the products was vacuum packaged in oxygen impermeable bags and stored at -20°C pending chemical analysis or their evaluation in a digestibility trial (see below). In another embodiment, specially in relation to the oils, 500 mg of santoquin were added per kg and then each lipid source was stored at 4-5°C in 1 L black plastic bottles.

#### EXAMPLE 10: Separation step

In preferred embodiments, the press liquid was separated into water and lipid fractions using an Alpha de Laval batch dairy centrifuge (Centrifuges Unlimited Inc., Calgary, Alberta). Then, the water fraction was condensed to about one third of its original volume using a steam jacketed bowl cooker.

#### EXAMPLE 11: Preparation of protein concentrates

Protein concentrates that are mostly based on protein from canola, soya, sunflower and hemp were prepared by hexane extracting the products that originated from the co-processing of 1:1 combinations of whole herring and each of the preceding oilseeds. In this regard, 200 g of each of the four protein products were extracted four times with hexane (5:1 v/w). During each extraction, the mixture was held for 30 min (stirred once after 15 min) before being filtered through Whatman No.1 filter paper in a Buchner funnel. Following hexane extraction, each protein product was placed on a tray that was lined with aluminum foil and then it was air-dried overnight. Then, each product was placed in the pellet cooler described in Example 9, where it was dried at about 70-80°C for 15 min to remove any residual traces of hexane.

#### EXAMPLE 12: *In vivo* protein digestibility experiments

In a preferred embodiment, the *in vivo* availability (digestibility) of protein in some of the test protein sources that were prepared by co-processing various proportions of whole herring with canola, soya, sunflower and hemp was determined using Atlantic salmon in sea water as the test animal. Two experiments were conducted and the experimental

conditions for each are provided in the table 1 below, wherein the flow rate of the oxygenated, filtered, ambient sea water was 6 - 8 L/min, feeding frequency was twice daily, ration was maximum (fish fed to satiation), and the photoperiod was natural.

**Table 1.**

Variable	Experiment 1	Experiment 2
Fish source	NorAm Aquaculture, Campbell River, BC	NorAm Aquaculture, Campbell River, BC
Range in initial mean weight (g)	76.6-85.8	54.2-61.6
Number of fish per tank	15	15
Tanks per diet	3	3
Stocking density (kg/m <sup>3</sup> )	<8.6	<6.2
Water temperature (°C)	8.9-9.1	9.0-9.5
Salinity (g/L)	29-31	28-30
Dissolved oxygen (mg/L)	8.5-9.4	7.5-9.0
Fecal collection period (days)	14	13

The design of the digestibility tanks and the fecal collection procedures have been described by Hajen *et al.* (1993a,b. Aquaculture 112: 321-348). The experimental diets consisted of 29.85% test protein product, 69.65% reference diet, and 0.5% chromic oxide as the indigestible marker. Table 2 outlined below provides the ingredient and proximate composition of the reference diet used in the digestibility experiments.

**Table 2.**

Ingredients	(g/kg; air-dry basis)
LT Anchovy meal	643.2
Blood flour; spray-dried	41.0
Pregelatinized wheat starch	80.9
Raw wheat starch	26.9
Vitamin supplement <sup>1/</sup>	18.9
Mineral supplement <sup>2/</sup>	18.9
Menhaden oil; stabilized <sup>3/</sup>	122.4
Soybean lecithin	9.46
Choline chloride (60%)	4.73
Vitamin C, monophosphate (42%)	3.38
Permapell	9.46
Finnstim <sup>TM</sup>	14.2
DL-methionine	1.51
Chromic oxide	5.00
Level of:	
Dry matter	924-926
Protein	452-453
Lipid	184
Ash	118-123

<sup>1</sup> The vitamin supplement provided the following amounts/kg of diet on an air-dry basis: vitamin A acetate, 4731 IU; cholecalciferol (D<sub>3</sub>), 2271 IU; DL- $\alpha$ -tocopheryl acetate (E), 284 IU; menadione, 17.0 mg; D-calcium pantothenate, 159.3 mg; pyridoxine HCl, 46.6 mg; riboflavin, 56.8 mg; niacin, 283.8 mg; folic acid, 14.2 mg; thiamine mononitrate, 53.0 mg; biotin, 1.42 mg; cyanocobalamin (B<sub>12</sub>), 0.085 mg; inositol, 378.5 mg.

<sup>2</sup> The mineral supplement provided the following (mg/kg diet on an air-dry basis): manganese (as MnSO<sub>4</sub> · H<sub>2</sub>O), 71.0; zinc (as ZnSO<sub>4</sub> · 7H<sub>2</sub>O), 85.2; cobalt (as CoCl<sub>2</sub> · 6H<sub>2</sub>O), 2.84; copper (as CuSO<sub>4</sub> · 5H<sub>2</sub>O), 6.62; iron (as FeSO<sub>4</sub> · 7H<sub>2</sub>O), 94.6; iodine (as KIO<sub>3</sub> and KI, 1:1), 9.46; fluorine (as NaF), 4.73; selenium (as Na<sub>2</sub>SeO<sub>3</sub>), 0.19; sodium (as NaCl), 1419; magnesium (as MgSO<sub>4</sub> · 7H<sub>2</sub>O), 378; potassium (as K<sub>2</sub>SO<sub>4</sub> and K<sub>2</sub>CO<sub>3</sub>, 1:1), 1419.

<sup>3</sup> Stabilized with 0.5 g santoquin/kg oil.

After adjustment of all experimental diet mashes to a moisture content of 9%, they were cold pelleted using a California model CL type 2 pellet mill. Diet particle size was adjusted to suit fish size. The reference and experimental diets that were used in the study were stored at 5°C in air-tight containers until required.

The reference and experimental diets (mixture of reference and test diet) and lyophilized fecal samples were analyzed for levels of moisture, protein and chromic oxide at the DFO, West Vancouver Laboratory (WVL) using the procedures described below. Subsequently, the digestibility coefficients for protein were determined for each diet according to Cho *et al.* (1985. *Finfish nutrition in Asia: methodological approaches to research and development*. IDRC Ottawa, Ont., 154p.). Then, the digestibility coefficients for each of the protein products themselves were calculated according to Forster (1999. *Aquaculture Nutrition* 5: 143-145).

The results of chemical analyses of the protein sources used in this study and of the

products derived from the co-processing of animal offals (herring or poultry offal) with canola, sunflower, soya and hemp treated as described above are presented in Tables 3-20. The results have been expressed on a dry weight basis and a lipid-free dry weight basis since the mechanical pressing of lipid from the cooked blends of offal and oilseed was variable and not complete. This is a function of the design of the presses and other conventional presses available in industry can be of higher efficiency.

Examples 13 to 16 outlined hereinafter give the results of chemical analyses performed on products obtained in accordance with the process of the invention from: canola and canola-based products, sunflower and sunflower-based products, soya and soya-based products, as well as hemp and hemp-based products. The chemical analyses were performed according to the following methods:

Concentrations of protein, moisture, and ash in the protein sources and products that were prepared as well as in all test diets and fecal samples were determined at the Department of Fisheries and Oceans, West Vancouver Laboratory (DFO-WVL) using the procedures described by Higgs *et al.* (1979. In J.E. Halver, and K. Tiews, eds. *Finfish Nutrition and Fishfeed Technology*, Vol. 2. Heenemann Verlagsgesellschaft MbH., Berlin, pp. 191-218).

Similarly, the fatty acid compositions of the cold pressed oils and animal feed grade oils stemming from the press liquids were determined at the same laboratory using the procedures of Silver *et al.* (1993. In S.J. Kaushik and P. Luquet, eds. *Fish nutrition in practice. IV<sup>th</sup> International Symposium on Fish Nutrition and Feeding*, INRA, Paris, pp. 459-468).

Moreover, the chromic acid concentrations in diets and lyophilized fecal samples were determined at the DFO-WVL using the methods of Fenton and Fenton (1979. *Can. J. Anim. Sci.*, 59: 631-634).

Concentrations of crude fibre (AOCS Official Method Ba 6-84), lipid (Troeng, S. 1955.

J.A.O.C.S. 32: 124-126), chlorogenic acid (capillary electrophoresis method developed by M. Marianchuk at the POS Pilot Plant Corp.) and sinapine (capillary electrophoresis method developed by P. Kolodziejczyk *et al.* at the POS Pilot Plant Corp.) in the oilseeds and test protein products as well as measurements of trypsin inhibitor (AOCS Official Method Ba 12-75 reapproved 1997) and urease (AOCS Official Method Ba 9-58 reapproved 1993) activities in soya and sunflower seeds and protein products were determined at the POS Pilot Plant Corp., Saskatoon, SK. according to the methods cited in the parentheses.

Determinations of the amino acid concentrations in the oilseeds and test protein products were conducted by AAA Laboratory, Mercer Island, WA, USA using the general procedures described by Mwachireya *et al.* (1999. Aquaculture Nutrition 5: 73-82).

Levels of phytic acid in all oilseeds and in the products derived from the co-processing of oilseeds and animal offal were determined by Ralston Analytical Laboratories, Saint Louis, MO using the procedures described by Forster *et al.* (1999. Aquaculture 179: 109-125).

Mineral concentrations in the oilseeds and the protein products were determined by Norwest Labs, Surrey, BC using plasma spectroscopy (Higgs *et al.*, 1982. Aquaculture 29: 1-31).

Concentrations of glucosinolate compounds (total of all the different types of glucosinolates) present in canola and canola-based products were measured by Dr. Phil Raney, of Agriculture & Agri-Food Canada, Saskatoon, SK according to the methods of Daun and McGregor (1981. Glucosinolate Analysis of Rapeseed (Canola). Method of the Canadian Grain Commission Revised Edn. Grain Research Laboratory, Canadian Grain Commission, Winnipeg, Manitoba, Canada).

Measurements of soy isoflavones namely, daidzein, glycitein, genistein, and saponins were conducted by Dr. Chung-Ja C. Jackson, of the Guelph Center for Functional Foods,

University of Guelph Laboratory Services and have been reported here as the total for the preceding compounds (the methodology in each case is the subject of a patent application and hence has not been published).

**EXAMPLE 13: Results obtained for canola and canola-based products**

Table 3 outlined below gives the percentages of extensively dehulled and partially dehulled Goliath canola seed and of hulls in relation to seed size after dehulling by Forsberg Incorporated, Thief River Falls, MN.

**Table 3.**

Seed size/fraction	Weight (kg)	%
Extensively dehulled; large <sup>1/</sup>	35.8	39.4
Extensively dehulled; small <sup>1/</sup>	10.8	11.8
Partially dehulled; large <sup>2/</sup>	20.4	22.4
Partially dehulled; small <sup>2/</sup>	14.3	15.7
Hulls; small <sup>3/</sup>	3.33	3.66
Hulls; large <sup>3/</sup>	6.49	7.13
Total	91.1	100

<sup>1/</sup> The extensively dehulled canola as identified visually by the lack of hulls in the material was used in the tests reported below (referred to as dehulled canola)

<sup>2/</sup> The partially dehulled canola could be subjected to further dehulling, directed into ruminant diets, and/or mixed at a low proportion with animal offal and then co-processed to create a nutritionally upgraded protein source for monogastrics.

<sup>3/</sup> The hulls contained little visible evidence of canola meats and had low density.

Table 4 gives the percentages of presscake and oil obtained after cold pressing raw, unde-hulled and micronized, dehulled Goliath canola seed using a laboratory scale Gusta

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press.

**Table 4.**

Fraction	Raw, dehulled canola seed	Micronized, dehulled canola seed
Presscake (%)	68.3	84.0
Oil (%)	31.7	16.0
Total	100	100

Table 5 sets out the initial ratios of water from endogenous and exogenous sources to oilseed lipid-free dry matter content and percentage yields (air-dry product, moisture-free product, and lipid-free dry weight product) from the co-processing of different blends of whole herring (WH) with dehulled, micronized (DC) and dehulled raw Goliath canola seed (URC).

**Table 5.**

Protein product <sup>1/</sup>	Initial ratio of hot water to oilseed lipid-free dry matter (w/w)	Air-dry product (%)	Moisture- free product (%)	Lipid-free dry product (%)
WH75DC25	5:1	29.4	27.0	19.4
WH50DC50	5:1	32.7	31.1	20.4
WH37.5DC62.5	5:1	34.8	31.8	20.0
WH75URC25	4.5:1	30.5	27.1	19.0
WH50URC50	5:1	30.9	29.8	21.3
WH25URC75	5:1	29.6	28.6	20.5

<sup>1/</sup> Numbers following WH, DC, and URC refer to initial percentages of these products in the herring/canola seed blends (canola seed was cold pressed to remove a significant portion of the oil and reduce the particle size of the starting material before blending with herring and santonin; 0.1g/kg of mixed product before water addition) before their co-processing using cooking temperatures of 90-93°C and drying temperatures of 77-83°C.

In Table 6, the concentrations of proximate constituents including crude fibre (CF) as well

as phytic acid (PA), total glucosinolates (TG), and sinapine in whole herring (WH), dehulled micronized cold pressed Goliath canola (DC), dehulled raw cold pressed Goliath canola (URC), and six protein products produced by the co-processing of different proportions of WH with either DC or URC (expressed on a dry weight basis, DWB or lipid-free dry weight basis, LFDWB) are provided. The composition of a seventh protein product that was produced by hexane extraction of WH50DC50 is also shown (WH50DC50-hexane) together with the apparent protein digestibility coefficients for some of the products (Atlantic salmon in sea water used as the test animal) is also provided.

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Table 6.

Parameter	WH	DC	URC	WH75 DC25	WH50 DC50	WH50 DC50 (hexane)	WH37.5 DC62.5	WH75 URC25	WH50 URC50	WH25 URC75
Dry matter (g/kg)	286	954	936	918	952	928	914	890	966	968
Protein (g/kg)	488	279	348	529	456	693	416	525	414	404
-LFDWB	870	515	469	735	696	724	662	748	578	564
Lipid (g/kg)	439	458	258	280	345	42.5	372	298	284	284
Ash (g/kg)	70.3	48	60.9	81	67.1	97.2	63	77.1	78.8	73.5
-LFDWB	125	88.6	82.1	113	102	102	100	110	110	103
CF (g/kg)	- <sup>1/</sup>	28.3	66.5	21	24.7	38.3	28.9	-	69.2	76.4
-LFDWB	-	52.2	89.6	29.2	37.7	40	46	-	96.6	107
PA (g/kg)	-	28.2	33.9	15.6	22.9	-	25.5	14.2	26	30.7
-LFDWB	-	52	45.6	21.6	35	-	40.6	20.2	36.3	42.9
TG (μmoles/g)	-	10.8	17.8	1.09	1.26	-	0.92	0.44	0.9	1.06
DWB										
-LFDWB	-	19.9	24	1.52	1.92	-	1.47	0.63	1.26	1.49
Sinapine(g/kg)	-	11.2	13.1	3.16	4.94	-	5.8	2.92	5.18	5.68
DWB										
-LFDWB	-	20.7	17.7	4.39	7.54	-	9.23	4.16	7.23	7.94
In vivo protein digestibility (%)	-	-	-	88.9	94.4	-	94.9	-	94.4	96.4

<sup>1/</sup> Not determined

Table 7 provides the concentrations of essential amino acids (% of protein) and selected minerals ( $\mu\text{g/g}$  of lipid-free dry matter) in whole herring (WH), micronized, dehulled, cold pressed Goliath canola (DC), undeulled, raw cold pressed Goliath canola (URC), and six protein products produced by the co-processing of different proportions of WH with either DC or URC. The amino acid and mineral concentrations in a seventh protein product, produced by hexane extraction of WH50DC50 are also shown (WH50DC50-hexane).

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Table 7.

Parameter	WH	DC	URC	WH75 DC25	WH50 DC50	WH50 DC50 (hexane)	WH37.5 DC62.5	WH75 URC2	WH50 URC5	WH25 URC75
A) Essential amino acids										
Arginine	6.66	7.09	-	7.23	7.44	7.5	6.93	-	-	-
Histidine	1.97	2.84	-	2.62	2.69	2.69	2.59	-	-	-
Isoleucine	4.56	4.28	-	4.81	4.78	4.71	4.51	-	-	-
Leucine	8.4	7.47	-	8.15	8.22	8.01	7.71	-	-	-
Lysine	5.47	3.87	-	4.92	4.85	7.01	4.4	-	-	-
Methionine + Cystine	3.97	4.55	-	4.54	4.63	4.25	4.47	-	-	-
Phenylalanine + Tyrosine	7.55	7.26	-	8.08	8.14	7.93	7.54	-	-	-
Threonine	4.97	4.62	-	4.83	4.89	4.73	4.61	-	-	-
Tryptophan	1.51	1.72	-	1.69	1.63	0.92	1.69	-	-	-
Valine	5.51	5.34	-	5.66	5.75	5.23	5.36	-	-	-
B) Minerals										
Calcium	30303	4061	5183	23905	14594	16202	12195	22088	14458	10244
Phosphorus	19073	18760	17278	23299	21971	23746	20384	21127	20675	20777
Magnesium	1961	7929	7631	4388	5934	6921	6098	4161	6289	8599
Sodium	5704	<100	<100	3026	1443	1598	1220	2081	772	495
Potassium	14260	18566	18142	12104	12348	14293	12544	10244	11234	12019
Copper	5.2	3.09	<1.00	15.4	6.09	12.0	8.36	11.4	10.7	9.81
Zinc	101	70.8	66.7	116	101	106	79.6	96.2	74.6	71.1

<sup>1/</sup> Not determined.

Table 8 sets out the percentages of selected fatty acids and of saturated, unsaturated, (n-6), (n-3) and n-3 highly unsaturated fatty acids (n-3 HUFA; 20:5 (n-3) + 22:6 (n-3)) in whole herring (WH), undehulled raw cold pressed Goliath canola (URC), and the press lipids resulting from the co-processing of different proportions of WH with DC or URC.

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Table 8.

Fatty acid	WH	URC	Lipid source							
			WH75 DC25	WH50 DC50	WH37.5 DC62.5	WH75 URC25	WH50 URC50	WH25 URC75		
18:1(n-9)	18.9	62.7	35.7	49.9	55.0	- <sup>1/</sup>	22	25.7		
18:2(n-6)	0.74	21.4	7.34	15.0	17.5	-	10.3	17		
18:3(n-3)	0.12	8.79	3.28	0.34	7.40	-	2.67	4.28		
20:5(n-3)	9.66	0.00	15.4	3.75	2.71	-	4.21	5.83		
22:6(n-3)	8.96	0.00	7.00	4.11	1.38	-	6.69	0		
Total	22.2	4.32	20.3	12.4	9.88	-	22.1	20.3		
Saturated										
Total	77.8	95.7	79.7	87.6	90.1	-	77.9	79.7		
Unsaturated										
Total (n-6)	4.82	21.7	8.56	15.5	17.9	-	14.1	20		
Total (n-3)	31.3	9.96	28.0	14.7	11.8	-	21.7	15.3		
Total n-3										
HUFA	18.6	0	22.4	7.86	4.09	-	10.09	5.813		

<sup>1/</sup> Not determined

**EXAMPLE 14: Results obtained for sunflower and sunflower-based products**

In Table 9, initial ratios of water from endogenous and exogenous sources to oilseed lipid-free dry matter and percentage yields (air-dry product, moisture-free product, and lipid-free dry weight product) from the co-processing of different blends of whole herring (WH) or poultry offal (PO) with dehulled, raw sunflower seed, batch 1 (DRSF<sub>1</sub>) or batch 2 (DRSF<sub>2</sub>) are provided.

**Table 9.**

Protein product <sup>1/</sup>	Initial ratio of hot water to oilseed lipid-free dry matter (w/w)	Air-dry product (%)	Moisture-free product (%)	Lipid-free dry product (%)
WH75DRSF <sub>1</sub> ,25	5:1	30.4	28.2	19.7
WH50DRSF <sub>1</sub> ,50	3:1	31.6	29.0	19.4
WH25DRSF <sub>1</sub> ,75	3:1	31.7	31.1	19.9
PO50DRSF <sub>2</sub> ,50	6:1	46.9	43.0	31.3

<sup>1/</sup> Numbers following WH, DRSF and PO refer to initial percentages of these products in the herring/sunflower seed and poultry/sunflower seed blends (sunflower seed was cold pressed to remove a significant portion of the oil and reduce the particle size of the starting material before blending with herring or poultry and santoquin; 0.1 g/kg of mixed product before water addition) before their co-processing using cooking temperatures of 90-93°C and drying temperatures of 77-83°C.

Table 10 gives the concentrations of proximate constituents including crude fibre (CF), phytic acid (PA), trypsin inhibitor activity (TI), urease activity (UA) and chlorogenic acid (CA) content in whole herring (WH), poultry offal (PO), dehulled, raw cold pressed sunflower, batch 1 (DRSF<sub>1</sub>), and five protein products produced by the co-processing of different proportions of WH or PO with either DRSF<sub>1</sub> or dehulled, raw cold pressed sunflower, batch 2 (DRSF<sub>2</sub>) (expressed on a dry weight basis, DWB or lipid-free dry weight basis, LFDWB). The composition of a sixth protein product that was produced by hexane extraction of WH50DRSF<sub>1</sub>,50 is also shown (WH50DRSF<sub>1</sub>,50-hexane) together with the apparent protein digestibility coefficients for some of the products (Atlantic salmon in sea water used as the test animal).

Table 10.

Parameter	WH	PO	DRSF <sub>1</sub>	WH75 DRSF <sub>1</sub> 25	WH50 DRSF <sub>1</sub> 50	WH50 DRSF <sub>1</sub> 50 (hexane)	WH25 DRSF <sub>1</sub> 75	PO50 DRSF <sub>2</sub> 50 <sup>2/</sup>
Dry matter (g/kg)	286	328	938	928	919	930	981	918
Protein (g/kg) -DWB	488	370	351	535	479	695	441	382
-LFDWB	870	673	594	766	715	718	689	525
Lipid (g/kg) -DWB	439	451	409	302	330	32.2	360	271
Ash (g/kg) -DWB	70.3	104	50.7	126	118	95.8	115	58.9
-LFDWB	125	189	85.8	181	176	99	180	80.8
CF (g/kg) -DWB	- <sup>1/</sup>	-	34	19.2	32.4	37.5	27.2	124
-LFDWB	-	-	57.5	27.5	48.4	38.8	42.5	170
PA (g/kg) -DWB	-	-	31.4	14.2	23.3	-	30.7	25.9
-LFDWB	-	-	53.2	20.4	34.8	-	47.9	35.6
Tl (TIA units/g) LFDWB	-	-	1603	1766	1730	-	1268	-
UA (ΔpH) -DWB	-	-	0.06	0.03	0.02	-	0.01	-
CA(g/kg) -LFDWB	-	-	14.9	2.6	5.65	-	8.58	6.22
In vivo protein digestibility (%)	-	-	-	3.72	8.43	-	13.4	8.53
	-	-	-	97.6	97.6	-	97.1	-

<sup>1/</sup> Not determined

<sup>2/</sup> DRSF<sub>2</sub> co-processed with PO was pressed, partially dehulled (58%) animal feed grade with a DM, protein, lipid, ash and crude fibre content (g/kg expressed on a dry weight basis except DM) of 918, 379, 211, 59.4, and 123, respectively.

Table 11 gives the concentrations of essential amino acids (% of protein) and selected minerals ( $\mu\text{g/g}$  of lipid-free dry matter) in whole herring (WH), poultry offal (PO), dehulled, raw, cold pressed sunflower, batch 1 (DRSF<sub>1</sub>), and four protein products produced by the co-processing of different proportions of WH or PO with either DRSF<sub>1</sub> or DRSF<sub>2</sub>. The concentrations in a fifth protein product, produced by hexane extraction of WH50DRSF<sub>1</sub>50, is also shown (WH50DRSF<sub>1</sub>50-hexane).

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Table 11

Parameter	WH	PO	DRSF, <sup>2/</sup>	WH75 DRSF <sub>1,25</sub>	WH50 DRSF <sub>1,50</sub>	WH50 DRSF <sub>1,50</sub> (hexane)	WH25 DRSF <sub>1,75</sub>	PO50 DRSF <sub>2,50</sub> <sup>3/</sup>
A) Essential amino acids								
Arginine	6.66	8.11	10.6	7.66	8.58	8.64	9.16	8.52
Histidine	1.97	1.91	2.59	2.34	2.42	2.40	2.41	2.56
Isoleucine	4.56	3.19	4.45	4.28	4.45	4.52	4.38	4.56
Leucine	8.40	5.88	6.32	7.16	6.96	7.11	6.57	6.95
Lysine	5.47	5.28	3.67	6.88	5.57	5.57	4.30	4.59
Methionine + Cystine	3.97	3.16	3.61	3.71	3.41	3.61	3.42	3.25
Phenylalanine +								
Tyrosine	7.55	5.45	7.66	7.46	7.65	7.82	7.55	7.72
Threonine	4.97	3.67	4.15	4.40	4.17	4.10	4.06	3.99
Tryptophan	1.51	0.75	1.28	1.27	0.78	1.27	1.03	1.40
Valine	5.51	4.03	5.19	5.09	5.29	4.86	5.08	4.81
B) Minerals								
Calcium	30303	- <sup>1/</sup>	1930	33810	15055	14999	10226	12420
Phosphorus	19073	-	22188	29950	25011	23221	23573	15843
Magnesium	1961	-	10805	4493	7503	7544	9987	4992
Sodium	5704	-	19.8	2223	1454	1378	836	852
Potassium	14260	-	23090	11085	14406	15110	15036	9894
Copper	5.20	-	39.1	21.6	36.5	28.9	37.0	39.9
Zinc	101	-	124	99.0	118	124	123	93.0

<sup>1/</sup> Not determined.

<sup>2/</sup> Values for essential amino acids were derived from unpressed DRSF<sub>1</sub>.

<sup>3/</sup> DRSF<sub>2</sub> co-processed with PO was partially dehulled (58%) animal feed grade with a DM, protein, lipid, ash and crude fibre composition (g/kg expressed on a dry weight basis except DM) of 918, 379, 211, 59.4, and 123, respectively.

In Table 12, percentages of selected fatty acids and of saturated, unsaturated, (n-6), (n-3) and n-3 highly unsaturated fatty acids (n-3 HUFA; 20:5 (n-3) + 22:6 (n-3)) in whole herring (WH), poultry offal (PO), dehulled, raw, cold pressed sunflower, batch 1 (DRSF<sub>1</sub>), and the press lipids resulting from the co-processing of different proportions of WH or PO with DRSF<sub>1</sub> or dehulled, raw, cold pressed sunflower, batch 2 (DRSF<sub>2</sub>).

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Table 12

Lipid source							
Fatty acid	WH	PO	DRSF <sub>1</sub>	WH75 DRSF <sub>1</sub> 25	WH50 DRSF <sub>1</sub> 50	WH25 DRSF <sub>1</sub> 75	PO50 DRSF <sub>2</sub> 50 <sup>2/</sup>
18:1(n-9)	18.9	39.9	9.39	21.6	18.2	- <sup>1/</sup>	17.9
18:2(n-6)	0.74	17.6	76.6	22.4	25.3	-	38.7
18:3(n-3)	0.12	2.56	0.11	4.28	0.42	-	0.80
20:5(n-3)	9.66	0.00	0.00	3.15	3.67	-	0.00
22:6(n-3)	8.96	0.00	0.00	6.04	7.87	-	0.00
Total							
Saturated	22.2	33.9	12.1	16.9	14.5	-	37.7
Total						-	
Unsaturated	77.8	66.17	87.9	83.1	85.5	-	62.3
Total (n-6)	4.82	17.8	76.6	29.5	36.3	-	38.7
Total (n-3)	31.3	2.56	0.12	19.2	17.7	-	0.80
Total n-3							
HUFA	18.6	0.00	0.00	9.19	11.5	-	0.00

<sup>1/</sup> Not determined.

<sup>2/</sup> DRSF<sub>2</sub> co-processed with PO was partially dehulled (58%) animal feed grade with a DM, crude protein, lipid, ash and crude fibre content (g/kg expressed on a dry weight basis except DM) of 918, 379, 211, 59.4, and 123, respectively.

EXAMPLE 15: Results obtained for soya and soya-based products

In Table 13, the initial ratios of water from endogenous and exogenous sources to oilseed, lipid-free dry matter and percentage yields (air-dry product, moisture-free product, and lipid-free dry weight product) from the co-processing of different blends of whole herring (WH) with dehulled, micronized (DSY) and undehulled raw soya seed (URSY).

Table 13.

Protein product <sup>1/</sup>	Initial ratio of hot water to oilseed lipid-free dry matter (w/w)	Air-dry product (%)	Moisture-free product (%)	Lipid-free dry product (%)
WH75DSY25	5:1	14.2	13.6	10.3
WH50DSY50	5:1	36.7	34.9	26.4
WH25DSY75	4:1	48.3	43.8	32.7
WH75URSY25	5:1	20.7	19.1	15.0
WH50URSY50	5:1	29.9	27.4	21.1
WH25URSY75	4:1	43.8	38.4	33.7

<sup>1/</sup> Numbers following WH, DSY and URSY refer to initial percentages of these products in the herring/soya blends (soya seed was ground to reduce the particle size of the starting material before blending with herring and santoquin; 0.1g/kg of mixed product before water addition) prior to their co-processing using cooking temperatures of 90-93 °C and drying temperatures of 77-83 °C.

Table 14 shows the concentrations of proximate constituents including crude fibre (CF) as well as phytic acid (PA), total saponins, total isoflavones (TIF), urease activity (UA), and trypsin inhibitor activity (TI) in whole herring (WH), dehulled, micronized, , soya (DSY), undehulled, raw soya (URSY), and six protein products produced by the co-processing of different proportions of WH with either DSY or URSY (expressed on a dry weight basis, DWB or lipid-free dry weight basis, LFDWB). The composition of a seventh protein product that was produced by hexane extraction of WH50DSY50 is also shown (WH50DSY50-hexane) together with the apparent protein digestibility coefficients for some of the products (Atlantic salmon in sea water used as the test animal).

Table 14

Parameter	WH	DSY	URSY	WH75 DSY25	WH50 DSY50	WH50 DSY50 (hexane)	WH25 DSY75	WH75 URSY25	WH50 URSY50	WH25 URSY75
Dry matter (g/kg)	286	921	897	956	950	936	907	921	916	878
Protein (g/kg)	-	488	334	526	531	647	507	497	429	388
DWB										
-LFDWB	870	522	434	696	701	668	680	633	557	504
Lipid (g/kg)	-	439	242	230	242	30.1	254	215	230	232
DWB										
Ash (g/kg)	-	70.3	50.3	57.1	77.2	59.4	71.2	85.8	66.4	56.8
DWB										
-LFDWB	125	66.4	74.2	102	78.4	73.4	70.0	109	86.2	74.0
CF (g/kg)	- <sup>1/</sup>	16.2	44.6	16.0	16.3	18.7	19.3	46.6	67.5	82.2
DWB										
-LFDWB	-	21.3	57.9	21.2	21.5	19.3	25.9	59.3	87.6	107
PA (g/kg)	-	14.9	20.0	9.87	11.9	-	12.9	12.5	15.9	17.2
DWB										
-LFDWB	-	19.6	25.9	13.1	15.7	-	17.3	15.9	20.7	22.4
Saponins (mg/g)	-	1.60	-	0.71	1.02	-	1.18	-	-	-
-DWB										
-LFDWB	-	2.11	-	0.94	1.35	-	1.58	-	-	-
TIF (µg/g)	-	2305	-	899	1402	-	1622	-	-	-
DWB										
-LFDWB	-	3041	-	1189	1850	-	2174	-	-	-
UA (ΔpH)	-	0.01	2.48	0.02	0.01	-	0.02	0.09	0.28	0.35
TI (TIA units/g)	-	7813	101563	871	1017	-	553	1902	8296	11138
LFDWB										
In vivo protein digestibility (%)	-	-	-	-	96.2	-	94.2	-	93.5	88.2

<sup>1/</sup> Not determined

Table 15 provides concentrations of essential amino acids (% of protein) and selected minerals ( $\mu\text{g/g}$  of lipid-free dry matter) in whole herring (WH), dehulled, micronized, soya (DSY), and three protein products produced by the co-processing of different proportions of WH with DSY. The concentrations in a fourth protein product, produced by hexane extraction of WH50DSY50, is also shown (WH50DSY50-hexane).

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Table 15

Parameter	WH	DSY	WH75 DSY25	WH50 DSY50	WH50 DSY50 (hexane)	WH25 DSY75
A) Essential amino acids						
Arginine	6.66	7.57	7.39	7.17	7.64	7.38
Histidine	1.97	2.48	2.45	2.42	2.49	2.47
Isoleucine	4.56	4.65	4.67	4.60	4.83	4.57
Leucine	8.40	7.53	7.66	7.48	8.00	7.58
Lysine	5.47	6.14	7.13	6.70	6.72	6.52
Methionine + Cystine	3.97	2.46	3.30	2.70	3.20	2.97
Phenylalanine +						
Tyrosine	7.55	8.56	8.21	8.27	8.78	8.47
Threonine	4.97	4.21	4.57	4.37	4.44	4.30
Tryptophan	1.51	1.45	1.38	1.31	1.20	1.35
Valine	5.51	4.54	5.26	5.04	4.79	4.99
B) Minerals						
Calcium	30303	2637	22138	14304	9958	8646
Phosphorus	19073	9339	19648	14998	11897	12385
Magnesium	1961	3638	2684	2597	2324	2971
Sodium	5704	<5.00	2228	1290	1157	668
Potassium	14260	27646	17157	16942	13769	17587
Copper	5.20	21.6	36.7	26.7	23.6	27.2
Zinc	101	57.3	75.3	65.5	65.6	67.8

Table 16 provides the percentages of selected fatty acids and of saturated, unsaturated, (n-6), (n-3) and n-3 highly unsaturated fatty acids (n-3 HUFA; 20:5 (n-3) + 22:6 (n-3)) in whole herring (WH), micronized, dehulled, soya (DSY), undehulled, raw soya (URSY), and the press lipids resulting from the co-processing of different proportions of WH with DSY or URSY.

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Table 16.

Lipid source										
Fatty acid	WH	DSY	URSY	WH75 DSY25	WH50 DSY50	WH25 DSY75	WH75 URSY25	WH50 URSY50	WH25 URSY75	
18:1(n-9)	18.9	17.8	17.4	17.9	18.86	15.8	18.4	14.5	13.1	
18:2(n-6)	0.74	57.5	57.2	6.39	10.1	22.8	8.24	13.0	25.2	
18:3(n-3)	0.12	9.79	10.2	2.67	2.19	4.38	2.99	2.79	4.60	
20:5(n-3)	9.66	0.00	0.00	11.2	6.92	5.58	10.4	7.85	5.17	
22:6(n-3)	8.96	0.00	0.00	8.55	8.11	6.77	9.10	8.33	6.29	
Total										
Saturated	22.2	13.4	13.3	22.1	25.7	22.8	21.0	26.4	26.8	
Total										
Unsaturated	77.8	86.6	86.7	77.9	74.3	77.2	79.0	73.6	73.2	
Total (n-6)	4.82	57.9	57.8	9.26	10.1	22.8	8.24	13.0	25.2	
Total (n-3)	31.3	10.0	10.4	29.3	25.6	24.5	32.7	27.5	22.1	
Total n-3										
HUFA	18.6	0.00	0.00	19.7	15.0	12.3	19.5	16.2	11.5	

Example 16: Results obtained for hemp and hemp-based products.

In Table 17, the initial ratios of water from endogenous and exogenous sources to oilseed lipid-free dry matter and percentage yields (air-dry product, moisture-free product, and lipid-free dry weight product) from the co-processing of different blends of whole herring (WH) with dehulled, sterilized (DHP) and unde-hulled sterilized hemp seed (UHP).

**Table 17.**

Protein product <sup>1/</sup>	Initial ratio of hot water to oilseed lipid-free dry matter (w/w)	Air-dry product (%)	Moisture-free product (%)	Lipid-free dry product (%)
WH75DHP25	5:1	3.04	2.93	2.80
WH50DHP50	4:1	20.4	19.9	15.1
WH25DHP75	3:1	37.3	32.6	23.2
WH75UHP25	5:1	15.0	14.7	11.9
WH50UHP50	5:1	36.9	36.4	31.4
WH25UHP75	4:1	40.3	39.7	34.2

<sup>1/</sup> Numbers following WH, DHP and UHP refer to initial percentages of these products in the herring/hemp blends (UHP seed was cold pressed to remove a significant portion of the oil and to reduce the particle size of the starting material before blending with herring and santoquin; 0.1g/kg of mixed product before water addition) prior to their co-processing using cooking temperatures of 90-93°C and drying temperatures of 77-83 °C.

Table 18 gives the concentrations of proximate constituents including crude fibre (CF) as well as phytic acid (PA) in whole herring (WH), dehulled, sterilized hemp (DHP), cold pressed unde-hulled, sterilized hemp (UHP), and six protein products produced by the co-processing of different proportions of WH with either DHP or UHP (expressed on a dry weight basis, DWB or lipid-free dry weight basis, LFDWB). The composition of a seventh protein product that was produced by hexane extraction of WH50DHP50 is also shown (WH50DHP50-hexane) together with the apparent protein digestibility coefficients for some of the products (Atlantic salmon in sea water used as the test animal).

Table 18.

Parameter	WH	DHP	UHP	WH75 DHP25	WH50 DHP50	WH50 DHP50 (hexane)	WH25 DHP75	WH75 UHP25	WH50 UHP50	WH25 UHP75
Dry matter (g/kg)	286	963	952	976	975	969	874	983	986	986
Protein (g/kg) -DWB	488	313	311	579	575	721	533	504	429	448
-LFDWB	870	632	399	765	757	746	750	625	498	520
Lipid (g/kg) DWB	-	439	505	221	240	33.4	289	193	138	138
Ash (g/kg) DWB	-	70.3	59.2	63.6	88.7	118	87.6	77.4	116	117
-LFDWB	125	120	81.6	131	117	122	123	95.9	135	136
CF (g/kg) -DWB	- <sup>1/</sup>	44.1	251	14.7	39.1	51.9	52.5	153	237	239
-LFDWB	-	89.2	322	19.4	51.4	53.7	73.9	189	275	277
PA (g/kg) -DWB	-	37.5	33.7	12.5	35.2	-	47.7	15.3	25.3	24.6
-LFDWB	-	75.7	43.3	16.5	46.3	-	67.1	18.9	29.3	28.6
In vivo protein digestibility (%)	-	-	-	-	96.1	-	99.9	-	-	-

<sup>1/</sup> Not determined

Table 19 shows the concentrations of essential amino acids (% of protein) and selected minerals ( $\mu\text{g/g}$  of lipid-free dry matter) in whole herring (WH), dehulled, sterilized hemp (DHP), and three protein products produced by the co-processing of different proportions of WH with DHP. or UHP. The concentrations in a fourth protein product, produced by hexane extraction of WH50DHP50, are also shown (WH50DHP50-hexane).

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Table 19

Parameter	WH	DHP	WH75 DHP25	WH50 DHP50	WH50 DHP50 (hexane)	WH25 DHP75
A) Essential amino acids						
Arginine	6.66	14.0	8.48	10.4	10.6	11.7
Histidine	1.97	2.81	2.53	2.58	2.62	2.71
Isoleucine	4.56	4.24	4.97	4.72	4.79	4.54
Leucine	8.40	6.72	8.32	7.70	7.90	7.31
Lysine	5.47	3.81	7.93	6.45	6.39	5.35
Methionine + Cystine	3.97	4.11	4.11	4.08	4.02	3.91
Phenylalanine + Tyrosine	7.55	8.41	8.62	8.48	8.68	8.52
Threonine	4.97	3.71	4.80	4.37	4.36	4.06
Tryptophan	1.51	0.40	0.41	0.75	1.39	0.75
Valine	5.51	4.97	5.58	5.37	5.19	5.20
B) Minerals						
Calcium	30303	1792	35867	16734	17616	7789
Phosphorus	19073	31048	29641	28340	27652	31219
Magnesium	1961	14202	3668	8772	8531	12375
Sodium	5704	37.8	2558	1646	1708	1162
Potassium	14260	18880	10882	11876	13559	14419
Copper	5.20	30.8	18.7	22.5	26.5	25.4
Zinc	101	169	101	125	141	154

Table 20 sets out the percentages of selected fatty acids and of saturated, unsaturated, (n-6), (n-3) and n-3 highly unsaturated fatty acids (n-3 HUFA; 20:5 (n-3) + 22:6 (n-3)) in whole herring (WH), dehulled, sterilized hemp (DHP), unde-hulled, sterilized hemp (UHP), and the press lipids resulting from the co-processing of different proportions of WH with DHP or UHP.

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Table 20

	Lipid source									
	WH	DHP	UHP	WH75 DHP25	WH50 DHP50	WH25 DHP75	WH75 UHP25	WH50 UHP50	WH25 UHP75	
Fatty acid										
18:1(n-9)	18.9	5.48	7.72	8.30	8.63	6.51	15.5	17.1	14.2	
18:2(n-6)	0.74	57.7	56.4	30.9	42.7	49.8	18.7	18.9	33.6	
18:3(n-3)	0.12	19.8	19.0	10.9	15.0	16.8	7.12	7.32	11.5	
20:5(n-3)	9.66	0.16	0.02	4.30	2.72	1.55	4.63	3.46	0.05	
22:6(n-3)	8.96	0.00	0.00	3.94	2.68	1.91	7.05	5.94	3.00	
Total										
Saturated	22.2	12.5	10.0	24.0	16.4	14.6	20.8	21.4	16.7	
Unsaturated	77.8	87.5	90.0	76.0	83.6	85.4	79.2	78.6	83.3	
Total (n-6)	4.82	57.7	60.6	31.6	42.8	49.9	21.8	20.9	36.5	
Total (n-3)	31.3	20.3	19.1	23.4	24.2	22.1	25.9	22.6	18.3	
Total n-3 HUFA	18.6	0.16	0.02	8.25	5.39	3.46	11.7	9.40	3.05	

The co-processing of animal offal with the foregoing oilseeds pretreated using the methods according to the present invention resulted in nutritionally upgraded protein sources suitable for use.

The yields of these protein sources were good for all canola and sunflower-based products and this was also true for the soya and hemp-based products when higher concentrations ( $\geq 50\%$  in initial mixture) of these treated oilseeds were used. All of the yields were likely underestimated of true values owing to the difficulty in quantitatively collecting all of the material from the drier portion of the fish meal machine.

The oilseed-based protein products contained high concentrations of protein that was highly bioavailable to salmon (generally 89% to 100% of the protein was noted to be digestible in Atlantic salmon held in sea water depending upon the source and percentage of the oilseed in the initial mixture of offal and oilseed and the pretreatment of the latter and the offal before their co-processing). Moreover, these protein products had significantly reduced concentrations of all heat labile and water soluble antinutritional factors except phytic acid relative to their respective initial levels in the oilseeds. Phytic acid was concentrated during the co-processing of offal with oilseed and the extent depended upon its initial concentration in the oilseed used in the process.

The fatty acid compositions of the animal feed grade lipid sources produced by the process largely reflected the fatty acid compositions and lipid levels contributed by the different proportions of the animal offal and oilseed used initially in the process. This provides considerable scope to produce specially designed lipid sources that are tailored to meet the fatty acid needs of various animal species.

The cold-pressing of oilseeds before they are blended with animal offal yielded high quality economically valuable human food grade oils whose fatty acid compositions can be varied, depending upon market requirements and the selection of the oilseed or combination of oilseeds that are used in cold pressing. The high value of the cold pressed oils which can be generated in greater quantities when dehulled seeds rather



than dehulled seeds are cold pressed will contribute to the overall economic viability of the co-processing of animal offals with oilseeds.

The hulls resulted from the dehulling of the oilseeds used in this study and the condensed solubles produced by co-processing animal offal(s) with oilseed(s) likely will be excellent organic fertilizer constituents. This is because they collectively contain soluble protein, some lipid and minerals and other components that can be degraded by aerobic or anaerobic bacterial processes into value-added fertilizer products making the overall process described herein economically viable.

The rapid heat treatment of oilseeds to inactivate enzymes like the protease inhibitors in soya and destruct heat labile antinutritional components coupled with the dehulling of oilseeds yield protein and lipid-rich products that potential can be used directly in high energy feeds such as those destined for aquatic species like salmon (salmon grower diets frequently contain 25-35% lipid on an air-dry basis and they are produced by extrusion processing technology).